MUSCLE BIOPSY IN THE STUDY OF ELECTROLYTE CHANGES, WITH PARTICULAR REFERENCE TO CHRONIC RENAL DISEASE*

By RENO R. PORTER, M.D., and (by invitation)
RICHARD A. NEUBAUER, M.D., ERNST FISCHER, M.D.,
and NELSON YOUNG, Ph.D.

RICHMOND, VIRGINIA

INTRODUCTION

In studies on the electrolyte phenomena in health and disease, serum determinations give direct information regarding both the vascular and extravascular extracellular fluid compartment since the vascular membrane is semipermeable and the concentration of electrolytes on both sides of this membrane are essentially the same. However, the cell wall is an entirely different sort of membrane, the exact nature of which is still obscure. As a consequence, the concentration of electrolytes is quite different on the two sides of this membrane and serum determinations give little direct information regarding the intracellular compartment. This compartment contains approximately two-thirds of the total body water, practically all of the body potassium, and the greatest percentage of magnesium, phosphate, protein and other substances found in the body.[1-4] The cell is the actual "living" portion of the body as compared to the extracellular portion which is primarily transport. Hence, observations on the fluid and electrolyte content of the intracellular compartment should give much more pertinent information as to the nature and effect of disease processes.

For this reason, the composition of the intracellular compartment has received increasing attention in recent years. Sporadic observations were made years ago, but only within the past

*From the Research Laboratories of the Department of Medicine, Medical College of Virginia.

These studies were supported by the Thalhimer Research Fund.

ten to fifteen years has a large body of evidence been accumulated. Balance studies on animals[5-7] and humans[8-14] have contributed much toward our knowledge of the alterations that take place in the intracellular compartment under certain circumstances. However, such studies are of limited value and must be interpreted with caution because their validity rests on certain assumptions which may or may not be true under all conditions. Much has been learned from direct tissue analyses in animals,[15-31] since this permits extensive observations to be made under a variety of abnormal circumsances. Such studies likewise have their limitations because of the questionable direct application to human disorders and the inability to produce certain human diseases in the animal. Human tissues have been studied post-mortem,[15, 31-33] but these observations have the disadvantage of showing only the end stage of disease and are limited in their interpretation by the uncertainty of the extent of post-mortem alterations.

Pilcher, Harrison,[34] and their co-workers, in 1931, reported the results of direct analysis of human muscle obtained during life by biopsy. They studied the changes in potassium only and used samples obtained from the gastrocnemius muscle taken under local anesthesia. Their "normal" control data was obtained from patients who had diseases other than the ones being studied, but nevertheless were in ill health and hence not normal. More recently, Mudge and Vislocky[35] reported their studies on antemortem human muscle. They obtained their muscle samples during general anesthesia and analyzed them for nitrogen, fat, chloride, sodium, and potassium. Again in this instance, however, abnormal individuals were used for the "control" observations.

We have been able to obtain data on the electrolyte and fluid composition of biopsied muscle taken from apparently healthy individuals during life. Since our values for the normal are not subject to the possible alterations associated with previously reported "normal" values, it seems worthwhile to report our findings at this time. In addition, we have accumulated similar data on individuals with chronic renal disease which show definite alterations when compared with the normal values. These alterations permit certain speculations as to the distribution of fluid and electrolytes in chronic renal disease. As have the authors previously mentioned, we have utilized muscle tissue because it is readily accessible during life. In addition, it is quite representative of the body as a whole since it contains, on a fat-free basis, 75% of the body water and (excluding the skeletal system which contains 80%) as much body ash as all other organs and tissues combined.

MATERIAL AND METHODS

Analyses were done on muscle samples obtained from nine individuals. The control observations were made on five apparently normal human male volunteers, who had no subjective or objective evidences of disease. Biopsies were taken from both the deltoid and gastrocnemius muscles in each subject. The abnormal studies were done on four cases having chronic renal disease. There were two cases of late stage chronic glomerulonephritis, one case of nephrotic stage of chronic glomerulonephritis, and one case of Kimmelstiel-Wilson disease. These cases varied in the presence or absence of azotemia and hypertension, but all had some degree of edema. The muscle biopsy in these cases was taken from the deltoid muscle because there was some degree of edema of the legs. There was no apparent edema at the site of the biopsy.

Our method[36] of muscle biopsy analysis is essentially similar to that used by others.[35, 37] The overlying skin and subcutaneous tissue were infiltrated with novocaine, taking care that no novocaine was put into the muscle. Approximately 2.5 gms. of muscle were excised and blotted free of excess blood and fluid with dry gauze. The specimen was placed in an air-tight specimen bottle and analytical procedures were done within an hour. Connective tissue and gross fat were removed and the muscle sample was minced with scissors until paste-like. Two portions were dried for 48 hours at 105°C for the determination of total fluid content. Fat was extracted with ether.[38] Muscle chloride was determined after digestion with nitric acid and silver nitrate by a modification of the Volhard method. Serum chloride was determined by the Schales method.[39] Nitrogen was determined by the micro-Kjeldahl method. Sodium and potassium were determined after digestion with nitric acid by Janke flame photometer, which contains an internal lithium standard.[40]

Certain comments should be made about these analytical procedures. Fat-free samples were not used in all cases, as will be noted. The chloride method used was found to be the most satisfactory, but it is a titration method using ammonium thiocyanate and the end-point is not as sharp as one would like. By our technique, the average percentile deviation from the mean value on duplicate determinations was ± 0.1% for water, ± 2.0% for chloride, ± 1.7% for potassium, and ± 3.4% for sodium.

From the basic data, certain other values can be calculated. The extracellular concentration of chloride, potassium and sodium were calculated from the serum concentrations by using the Donnan factor of 1.04. The quantity of extracellular water was computed in two ways. This and other calculations will be discussed subsequently.

Results

Table I shows the data obtained by analysis of specimens of deltoid muscle taken from five normal individuals. As mentioned previously, samples were taken at the same time from the gastrocnemius muscle, but only the deltoid values are given since the abnormal values represent deltoid muscle. There is a normal variation between different muscle groups, so it seems proper to compare the same muscle in the normal and abnormal cases. The first group of figures represents the values for chloride, potassium and sodium in meq. per Kg. of dry muscle by direct analysis. There is considerable variation in these figures. Specimens 7, 17 and 18 are in rather close agreement, while specimens 8 and 11 deviate considerably from the other three. Not being sure whether these represent technical errors or the wide range of normal, they are all included and used as the basis for computing the

TABLE I

NORMAL DELTOID MUSCLE

No.	*Dry Muscle—meq/kg*			*Whole Muscle—meq/kg*		
	Cl—	*K+*	*Na+*	*Cl—*	*K+*	*Na+*
*7	119	396	171	28.4	91.4	39.4
8	107	359	175	27.4	91.9	44.8
*11	151	390	170	35.3	91.0	39.6
*17	127	406	153	28.6	91.8	34.6
*18	125	401	156	28.9	93.0	36.1
Avg.	126	390	165	29.7	91.8	38.9

*Fat-free analysis.

average figures. As will be seen later, the figures for abnormal muscle in most instances are beyond the wide range of normal, as indicated by these somewhat scattered figures. The average values are 126 meq. of chloride, 390 meq. of potassium, and 165 meq. of sodium per Kg. of dry muscle. The second group of figures are the values for whole muscle obtained by calculating the dry weight figures on the basis of the undried muscle weight, the original state in which the muscle was obtained. Since these are based on the original figures, there is a similar variation modified to some extent by the percentage of water in each sample. The averages for whole muscle are 29.7 meq. of chloride, 91.8 meq. of potassium, and 38.9 meq. of sodium per Kg.

The chloride and sodium contents of whole muscle are approximately the same as those reported by Mudge and Vislocky;[35] the potassium value is somewhat higher than these authors found. When the average potassium value is calculated on the basis of percentage by weight, it is found to be 0.359% in our cases. This agrees rather closely with the average value of 0.345% reported by Pilcher and Harrison,[34] 0.349% reported by Norm[32] in 1929, and 0.365% reported by Katz in 1896. It differs considerably from the value of 0.263% reported by Lemotte[15] in 1928.

These comparisons, however, are of limited value. In the first place, as pointed out above, all previously reported "normal" values were obtained from abnormal individuals and/or under abnormal circumstances, which casts doubt on their normalcy. In the second place, there is a normal variation between muscle groups, so that one should not attempt to compare values from different muscle groups. We feel that our values are more likely to approximate the actual normal muscle content of these electrolytes. We think this is particularly true of the potassium content of whole muscle because of the very close agreement in all five samples. The fact that his close agreement occurs in the whole muscle while there is more variation in the dry muscle values is due to the variation in water content. The lowest dry muscle potassium is found in the specimen having the least water, and vice versa.

Table II shows the fluid distribution within these muscle samples and the concentration of potassium and sodium in the intracellular fluid. As in the previous table, there is considerable variation in these results and, likewise, specimens #8 and #11

TABLE II

NORMAL DELTOID MUSCLE
(Based on "Chloride" Space)

No.	*Fluid L/Kg. Whole Muscle*				*I.C.W. meq/L*		
	Total	*E.C.W.*	*I.C.W.*	*%E.C.W.*	K^+	Na^+	$Na + K+$
*7	.770	.267	.503	34.7	180	10.3	190
8	.744	.251	.493	33.7	184	21.3	205
11	.767	.334	.433	43.5	207	0	207
*17	.774	.272	.502	35.1	181	0	181
*18	.768	.278	.490	36.2	188	0	188
Avg.	.765	.280	.484	36.6	188	6.3	194

*Fat-free analysis.

deviate considerably from the other three. The total fluid is determined by the difference in weight between the original whole muscle sample and the weight after drying. The other values in this table are computed from the original data and are based on the concept that the "chloride space" is a measure of the quantity of extracellular fluid (ECW).[4, 41-45] This concept is based on the assumption that chloride is not present within the cell and hence is entirely extracellular. (There is some doubt as to the validity of this concept which is discussed in a later section.) By dividing the amount of chloride per Kg. of muscle in the sample by the extracellular concentration of chloride, we can obtain the amount of extracellular fluid per Kg. of muscle. Subtracting this from the total fluid gives the volume of the intracellular fluid (ICW) per Kg. of whole muscle. There is some variation in the values, particularly in samples #8 and #11. On the average, each Kg. of muscle contains .765 liter of fluid, of which .280 liter is extracellular and .484 liter is intracellular. The percentage of total water which is extracellular averages 36.6%.

The amount of sodium and potassium in the ECW is obtained by multiplying the extracellular concentration by the volume of extracellular fluid. Subtracting this value from the total quantity in the muscle sample gives the amount of the electrolyte in the intracellular fluid. Dividing this by the quantity of intracellular fluid gives the concentration shown in the second portion of the table. The values for the intracellular concentration of potassium are considerably higher than those previously reported in humans, but we are inclined to believe that our figures more nearly approximate the true value.

The values for intracellular sodium are not so reliable and, as is obvious from our figures, there is considerable doubt as to their validity. In other words, it is improbable for one normal person to have 21.3 meq/L of sodium within his cells and another normal person to have none. This is a mathematical phenomena which affects sodium particularly because the quantity of extracellular sodium is so much greater than the quantity of intracellular sodium, which is quite small. Consequently, any slight technical error in determining the volume of extracellular fluid, which includes not only the titration of chloride but also the validity of the "chloride space," will cause significant alterations in the quantity of intracellular sodium. In addition, there exists a source of error in the determination of sodium which gives a percentage deviation from the mean of 3.4%, which may amount to as much as 3 meq. per Kg. of whole muscle. Therefore, we doubt the accuracy of the individual figures for intracellular concentrations of sodium, but have presented them for the sake of completeness and subsequent comparison. The average value of 6.3 is close to what the normal is considered to be, but such an average is certainly unsound mathematically.

In order to present this data in a more graphic form, we have constructed a schematic chart to indicate the probable distribution of fluid and electrolytes within the body as a whole (Fig. 1). In this schema we have utilized data obtained from a variety of sources,[1, 2, 3, 46] substituting our values in those instances where applicable. We are making the assumption that the values we found in a small muscle sample apply to the entire body. This of course is not completely true because even in muscle tissue itself there is some variation in the electrolyte values between different muscle groups. This difference may be as much as 15% for chloride, 10% for sodium, and 5% for potassium. However, the variations are relatively insignificant when compared with the order of changes seen in abnormal conditions. In addition, as mentioned previously, muscle comprises a large proportion of the body water and, excluding bone, a considerable portion of the body ash. The values obtained in a sample of muscle represent, therefore, the situation in a large proportion of the entire body and, perhaps, may be representative of the whole body. The volume of fluid is shown by the horizontal line and indicates, for a 70 Kg. man, about 3 liters (5% of body weight) of intravascular extracellular fluid, about 11 liters (15% of body weight) of interstitial or extravascular extracellular fluid, and approximately 35 liters (50% of body weight) of intracellular fluid. Recently, there has appeared in the literature[47-49] rather

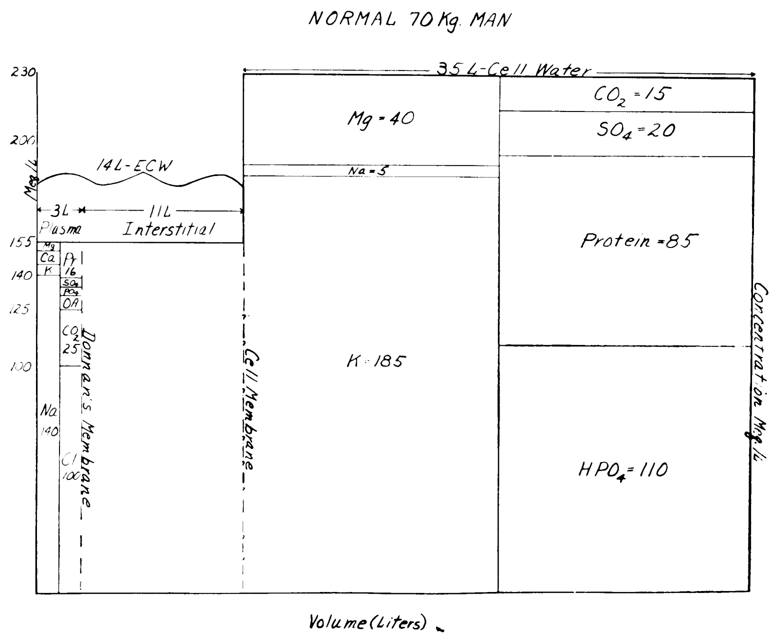

FIGURE 1. Schema illustrating the normal quantity and distribution of fluid and electrolytes throughout the body compartments. The horizontal line represents volume and the vertical line represents concentration.

convincing evidence that the extracellular space is closer to 16% rather than the 20% as pictured here. This would make the extracellular volume 11.2 liters instead of 14 liters and move the line indicating the cell wall slightly to the left. This would not alter the chart otherwise, and, for the sake of comparison, we will assume this chart to be approximately correct.

The concentration of electrolytes in the different compartments is indicated by the vertical line. The extracellular electrolytes are shown in the left portion of the figure. The concentrations shown are accepted values. The intracellular electrolytes are shown on the right. We have used our value of 185 for potassium which is about 30 meq. higher than the 155 which is generally used. The sodium is an accepted value and approximates the average we obtained. We have no figures ourselves on magnesium, but reported values of approximately 40 appear to be valid. Because of the higher value for potassium and no known diminution in any other accepted cation value, our observations raise the total base concentration within the cell from the usually accepted 200 to 230. This has given trouble on the anion side because we have no observations on these to indicate any specific increase in values. Thus, we have had to raise arbitrarily the "acid acting" portion of protein from accepted 65 to 85 and phosphate from 100 to 110. These alterations in accepted values for protein and phosphate were necessary for balance, but they may not be justified. However, our interest is primarily in sodium and potassium and the other alterations, for which we have no experimental proof, merely complete a possible picture.

TABLE III

DELTOID MUSCLE IN CHRONIC RENAL DISEASE

No.	*Dry Muscle—meq/kg.*			*Whole Muscle—meq/kg.*		
	Cl—	*K+*	*Na+*	*Cl—*	*K+*	*Na+*
4	194	439	344	38.5	87.0	68.2
*14	176	423	208	38.4	92.3	45.4
*15	247	382	256	53.9	83.6	55.9
*20	248	390	239	51.9	81.7	50.2
Avg.	216	408	262	45.7	86.2	54.9

*Fat-free analysis.
#4Late stage of chronic glomerulonephritis and pyelitis.
#14 Kimmelstiel-Wilson Disease.
#15 Nephrotic stage of chronic glomerulonephritis.
#20 Late stage of chronic glomerulonephritis.

The electrolyte and fluid data obtained in the four cases of chronic renal disease are shown in Tables III and IV. The chloride content of whole muscle in chronic renal disease is increased considerably, with values in each case well above the normal range and the average increased 54%. Potassium is slightly below normal in all cases except one, as is the average value. Sodium is increased significantly, with individual values being above the range of normal and the average showing a 41% increase over the normal.

TABLE IV

DELTOID MUSCLE IN CHRONIC RENAL DISEASE
(Based on "Chloride" Space)

No.	*Fluid L/Kg. Whole Muscle*				*I.C.W. Meq/L*		
	Total	*E.C.W.*	*I.C.W.*	*%E.C.W.*	*K+*	*Na+*	*Na + K+*
4	.802	.388	.414	48.4	204	48.1	252
*14	.782	.360	.422	46.0	214	0	214
*15	.782	.494	.288	63.2	285	0	285
*20	.791	.455	.336	57.5	236	0	236
Avg.	.789	.424	.365	53.8	235	12.0	247

*Fat-free analysis.

Total fluid is slightly increased, and there is a striking alteration in the distribution of this fluid when calculated on the basis of "chloride space." Extracellular water is considerably increased and intracellular water is decreased. This increases the percentage of extracellular water to average value of 53.8%. There is an increase in the concentration of potassium and sodium within the cell. Fig. 2 shows these alterations in graphic form. Since the increase in potassium and sodium is approximately 25%, we have arbitrarily increased the other cations and anions 25% to balance the concentration. This seems justified by the fact that there is a calculated reduction in intracellular water of approximately 25%.

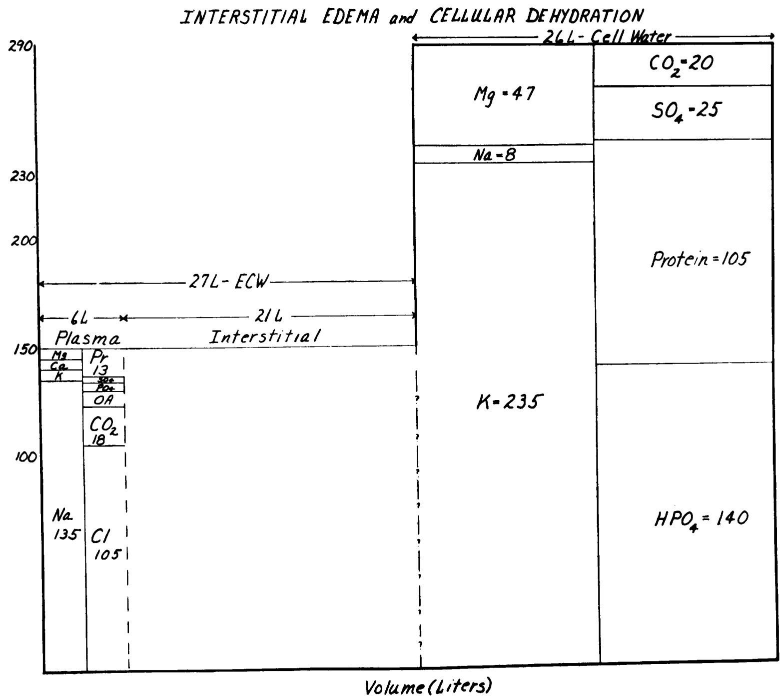

FIGURE 2. Quantity and distribution of fluid and electrolytes throughout the body compartments in chronic renal disease. Data obtained by calculations based on "chloride space" as a means of extracellular fluid.

DISCUSSION

Thus, in chronic renal disease we have found that there is a considerable increase in chloride and sodium, a slight decrease in potassium, and a slight increase in total fluid in whole muscle on direct analysis. Calculations based on the assumption that the "chloride space" is a measure of the extracellular fluid indicate further that there is also extracellular edema and intracellular dehydration, with increased concentrations of intracellular potassium and sodium.

Pilcher and Harrison[34] found a more marked decrease in the potassium content of muscle in one case of renal edema, but the sample was taken from an obviously edematous area. In studying cases with cardiac as well as renal edema they found that the decrease in potassium was somewhat proportional to the degree of edema but was better correlated with the duration of edema. We have no specific data on this point. Mudge and Vislocky[35] in three cases of renal acidosis found an increase in sodium and chloride, a marked reduction in potassium, and a slight decrease in total water in whole muscle. Their calculated data indicates decrease in intracellular potassium and an increase in intracellular sodium. The distribution of fluid did not differ significantly from the control values.

There are a number of points that make us question the likelihood of this calculated situation of extracellular edema and intracellular dehydration. The first is the possible unreliability of the "chloride space" as a measure of extracellular fluid under these circumstances. We are now dealing with an abnormal condition in which it is probable that chloride is intracellular to some extent and hence not confined exclusively to the extracellular compartment. The second unlikely factor is the existence of the considerable cellular dehydration shown here. Such dehydration would seem detrimental to the cells and would be corrected by the body if it were at all possible. Since there is ample fluid in the extracellular space, and the cell wall, so far as we know, is freely permeable to water, there appears to be no reason for the body to permit such dehydration. As this situation of extracellular edema and intracellular dehydration appears to be unlikely, the question of some other distribution of fluid and electrolytes in chronic renal disease is raised. The crux of the problem is the determination of what portion of the total fluid is extracellular and what is intracellular. Unfortunately, our present technique does not permit what we would consider a reliable determination of this under pathological conditions, so that we cannot be sure what the true picture is.

However, one other possibility presents itself and that is that there may be a normal or near-normal distribution of fluid between the extracellular and intracellular compartments. With this assumption, the extracellular fluid can be calculated as 36.6% of the total fluid and a different sort of situation obtained. Such a calculation will reduce the amount of extracellular edema, which may not appear justified in patients having some degree of edema. As has been mentioned, there was no obvious edema at the site of muscle biopsy, but that does not exclude subclinical edema. Acutally, the muscle samples were edematous since the volume of total fluid was increased over the normal. This second way of calculation merely rearranges the distribution of this fluid. Actually, there is an increase in extracellular fluid, which may be the situation in early "subclinical" edema.

Table V shows the comparative data of normal, chronic renal disease based on "chloride space," and chronic renal disease based on a normal percentage of extracellular fluid. With the chronic renal disease values so "corrected," there is a slight increase in total, extracellular and intracellular water. The intracellular compartment contains a considerable quantity of chloride,

TABLE V

DELTOID MUSCLE

	Total Fluid	*E.C.W.*	*I.C.W.*	% *E.C.W.*	*I.C.W. meq/L*		
					Cl−	*K+*	*Na+*
Normal	.765	.280	.484	36.6	0	188	6.3
Renal Dis. (C*l* space)	.789	.424	.365	53.8	0	235	12.0
Renal Dis. (corrected)	.789	.289	.500	36.6	29.3	169	35.2

Comparison of average values for fluid distribution and intracellular concentration of chloride, potassium and sodium. The values for normal and renal disease (Cl space) have been calculated on the basis that the chloride space is a measure of the extra-cellular fluid. The values for renal disease (corrected) have been calculated on the basis that the percentage of extracellular fluid is normal.

an increased concentration of sodium, and a slightly reduced concentration of potassium. Figure 3 is a graphic presentation of this possible situation. The values for the other intracellular cations and anions have been adjusted arbitrarily to fit the picture.

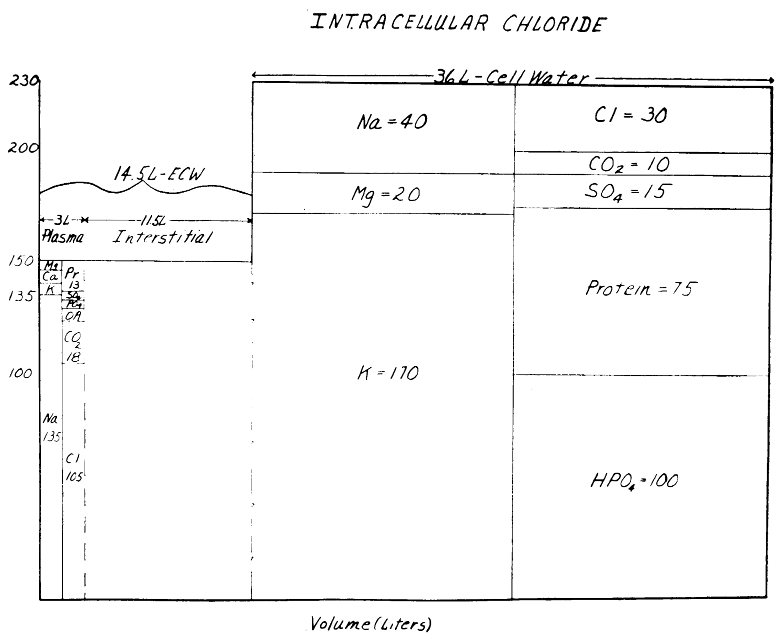

FIGURE 3. Quantity and distribution of fluid and electrolytes throughout the body compartments in chronic renal disease. Calculations based on the normal percentage of extracellular fluid.

This situation has several points in its favor which make it a definite possibility. In the first place, it eliminates the questionable chloride space as a measure of extracellular fluid. In the second place, there is no cellular dehydration, which we feel is an improbable or unlikely state of affairs. Finally, balance studies[50] on similar patients under treatment with sodium and potassium acetate indicate that there is a large negative chloride balance which cannot be accounted for as coming from the extracellular fluid. This suggests that there is an increased body store of chloride in these conditions which may very well be within the cell.

It is impossible, of course, for us to say which of these two possibilities, intracellular dehydration or intracellular chloride, is the actual situation existing in chronic renal disease. It is our belief that the true picture lies somewhere in between these two extremes. Certain factors make it appear that the probable situation is predominantly that of intracellular chloride.

Conclusions

1. A procedure for the determination of water, fat, nitrogen, chloride, sodium and potassium in a biopsied piece of muscle has been described.

2. Normal values, as determined by this procedure in a group of apparently healthy human volunteers, have been presented.

3. Similar observations on subjects with chronic renal disease and varying degrees of edema show a slight increase in total fluid, a slight decrease in potassium, and a considerable increase in sodium and chloride in whole muscle.

4. Calculations from this data show that there is either (a) extracellular edema and intracellular dehydration, or (b) an intracellular migration of chloride, an increase in intracellular sodium and a decrease in intracellular potassium.

5. We are unable to determine accurately the actual situation but believe that it lies somewhere in between these two extremes. It is suggested that the situation is predominantly, if not entirely, that characterized by the presence of chloride within the cell.

6. No attempt has been made to explain the mechanism by which these alterations were brought about.

Acknowledgements

We are indebted to Miss Lucy Harvie, Miss Alice Goodman, and Miss Elizabeth Pahnelas for technical assistance.

BIBLIOGRAPHY

1. PETERS, J. P.: Body Water. Charles C. Thomas, 1935.

2. SHOHL, A. T.: Mineral Metabolism. Reinhold Publishing Co., New York, 1939.

3. DARROW, D. C.: Medical Progress: Body Fluid Physiology; The Relation of Tissue Composition to Problems of Water and Electrolyte Balance. N.E.J. Med. *233*:91, 1945.

4. PETERS, J. P.: Transfer of Water and Solutes in the Body. The Harvey Lectures, 1937-38, p. 112.

5. DARROW, D. C., and YANNET, H.: The Changes in the Distribution of Body Water Accompanying Increase and Decrease in Extracellular Electrolytes. J.C.I. *14*:266, 1935.

6. ELKINTON, J. R., and TAFFEL, M.: Prolonged Water Deprivation in the Dog. J.C.I. *21*:787, 1942.

7. ELKINTON, J. R., and WINKLER, A. W.: Transfers of Intracellular Potassium in Experimental Dehydration. J.C.I.. *23*:93, 1944.

8. GAMBLE, J. L., ROSS, G. S., and TISDALL, F. F.: The Metabolism of Fixed Base During Fasting. J. Biol. Chem. *57*:633, 1923.

9. ELKINTON, J. R., WINKLER, A. W., and DANOWSKI, T. S.: Inactive Cell Base and the Measurement of Changes in Cell Water. Yale J. Biol. Med. *17*:384, 1944.

10. ELKINTON, J. R., WINKLER, A. W., and DANOWSKI, T. S.: Transfer of Cell Sodium and Potassium in Experimental and Clinical Conditions. J.C.I. *27*:74, 1948.

11. DARROW, D. C.: Medical Progress: Body Fluid Physiology: The Relation of Tissue Composition to Problems of Water and Electrolyte Balance. N.E.J. Med. *233*:91, 1945.

12. DARROW, D. C.: Congenital Alkalosis with Diarrhea. J. Ped. *26*:519, 1945.

13. DARROW, D. C.: The Retention of Electrolytes During Recovery from Severe Dehydration Due to Diarrhea. J. Ped. *28*:515, 1946.

14. GREEMAN, L., MATEER, F. M., GOW, R. C., PETERS, J. H., and DANOWSKI, T. S.: Some Observations on the Development of Hypokalemia During Therapy for Diabetic Acidosis in Juvenile and Young Adult Subjects. J.C.I. *28*:409, 1949.

15. LEMATTE, L., BOINOT, G., and KAHANE, E.: Mineral Content of Human and Animal Tissues. Bull. Soc. Chem. Biol. *10*:553, 1928.

16. Harrison, H. E., Darrow, D. C., and Yannet, H.: The Total Electrolyte Content of Animals and Its Probable Relation to the Distribution of Body Water. J. Biol. Chem. *113*:515, 1936.

17. Amberson, W. R., Nash, T. P., Mulder, A. G., and Bennis, D.: The Relationship Between Tissue Chloride and Plasma Chloride. Am. J. Physiol. *122*:224, 1938.

18. Heppel, L. A.: The Electrolytes of Muscle and Liver in Potassium Depleted Rats. Am. J. Physiol. *127*:385, 1939.

19. Manery, J. F., and Hastings, A. B.: The Distribution of Electrolytes in Mammalian Tissues. J. Biol. Chem. *127*:657, 1939.

20. Heppel, L. A.: The Diffusion of Radioactive Sodium into the Muscles of Potassium Deprived Rats. Am. J. Physiol. *128*:449, 1940.

21. Yannet, H., and Darrow, D. C.: The Effect of Depletion of Extracellular Electrolytes on the Chemical Composition of Skeletal Muscle, Liver and Cardiac Muscle. J. Biol. Chem. *134*:721, 1940.

22. Miller, W. C., and Darrow, D. C.: Relation of Muscle Electrolytes to Alterations in Serum Potassium to Toxic Effects of Injected Potassium Chloride. Am. J. Physiol. *130*:747, 1940.

23. Wallace, W. M., and Hastings, A. B.: The Distribution of Bicarb Ion in Mammalian Muscle. J.B.C., *144*:637, 1942.

24. Wilde, W. C.: The Chloride Equilibrium in Muscle. Am. J. Physiol. *143*:666, 1945.

25. Darrow, D. C.: Changes in Muscle Composition in Alkalosis. J.C.I. *25*:324, 1946.

26. Danowski, T. S., Hald, P. M., and Peters, J. P.: Sodium, Potassium and Phosphates in the Cells and Serum of Blood in Diabetic Acidosis. Am. J. Physiol. *149*:667, 1947.

27. Danowski, T. S., Hald, P. M., and Peters, J. P.: Distribution and Movements of Inorganic Phosphate Between Cells and Serum of Human Blood. Am. J. Physiol. *149*:678, 1947.

28. Darrow, D. C., Schwartz, R., Iannucci, J. F., and Coville, F.: The Relation of Serum Bicarbonate Concentration to Muscle Composition. J.C.I. *27*:198, 1948.

29. Darrow, D. C., Schwartz, R., Iannucci, J. F., and Coville, F.: The Relation of Serum Bicarbonate Concentration to Muscle Composition. J.C.I. *27*:198, 1948.

30. Muntwyler, Edward, Griffin, Grace E., Samuelson, George S., and Griffith, Lois E.: Federation Proceedings, *8*:231, 1949.

31. Fox, C. L.: Personal Communication.

32. Norm, M.: Scand. Arch. of Physiol. *55*:162, 1929.

33. Harrison, T. R., Pilcher, C., Ewing, G.: Studies in Congestive Failure. IV. The Potassium Content of Skeletal and Cardiac Muscle J.C.I. *8*:325, 1930.

34. Pilcher, C., Calhoun, J. A., Cullen, G. E., Harrison, T. R.: Studies in Congestive Heart Failure. Potassium Content of Skeletal Muscle Obtained by Biopsy. J.C.I. *9*:191, 1931.

35. Mudge, G. H., and Vislocky, K.: Electrolyte Changes in Human Striated Muscle in Acidosis and Alkalosis. J.C.I. *28*:482, May 1949.

36. Neubauer, R. A., Fischer, E., Young, N. F., Blades, J. F., and Porter, R. R.: Electrolyte Composition of Skeletal Muscle Obtained by Biopsy. Preliminary Report of Normal Values. To be published.

37. Lowry, O. H., and Hastings, A. B.: Histo-Chemical Changes Associated with Aging; Methods and Calculations. J. Biol. Chem. *143*:257, 1942.

38. Hastings, A. B., and Eichelberger, L.: I. Exchange of Salt and Water Between Muscle and Blood; Effect of an Increase in Total Body Water. Produced by Intravenous Injection of Isotonic Salt Solutions. J. Biol. Chem. 1937, *117*:73.

39. Schales, O., and Schales, S. S.: A Simple and Accurate Method for the Determination of Chloride in Biological Fluids. J. Biol. Chem. *140*:530, 1941.

40. Berry, J. W., Chappel, D. G., and Barnes, R. B.: Improved Method of Flame Photometry. Ind. and Engineer Chem. (Analytical), *18*:19, 1946.

41. Fenn, W. O.: Electrolytes in Muscle. Physiol. Rev. *16*:450, 1936.

42. Eggleton, M. G., Eggleton, P., and Hamilton, A. M.: Distribution of Chloride in Frog's Skeletal Muscle Immersed in Saline Solution. J. Physiol. 90:167, 1937.

43. Peters, J. P.: Transfer of Water and Solutes in the Body. The Harvey Lectures, 1937-38, page 112.

44. Gersh, I.: Anat. Record *70*:311, 1938.

45. Dean, R. B.: The Determination of Chloride in Single Isolated Muscle Fibers. J. Biol. Chem. *137*:113, 1941.

46. Gamble, J. L.: Chemical Anatomy, Physiology and Pathology of the Extracellular Fluid. Harvard University Press. 1947.

47. BOYLE, P. J., and CONWAY, E. J.: Potassium Accumulation in Muscle and Associated Changes. J. Physiol. *100*:1, 1941.

48. DARROW, D. C.: Tissue Water and Electrolytes. Ann. Rev. of Physiol. *6*:95, 1944.

49. SCHWARTZ, I. L., SCHACHTER, D., and FREINKEL, N.: The Measurement of Extracellular Fluid in Man by Means of Constant Infusion Technique. J.C.I. *28*:1117, 1949.

50. PORTER, R. R., NEUBAUER, R. A., FISCHER, E., and YOUNG, N. F.: Electrolyte Therapy in Chronic Renal Disease. To be published.